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Impact of temperature increase and acidification on growth and the reproductive potential of the clam *Ruditapes philippinarum* using DEB☆

F. Maynou *, E. Galimany, M. Ramón, M. Solé

Institut de Ciències del Mar, CSIC, Psg. Marítim de la Barceloneta 37-49, 08003, Barcelona, Spain

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ABSTRACT

We built a simulation model based on Dynamic Energy Budget theory (DEB) to assess the growth and reproductive potential of the Manila clam *Ruditapes philippinarum* under different temperature and pH conditions, based on environmental values forecasted for the end of the 21st c. under climate change scenarios. The parameters of the DEB model were calibrated with the results of seasonal growth experiments under two levels of temperature (ambient and plus 2–3 °C) and three levels of pH (8.1 used as control and 7.7 and 7.3 representing acidification). The results showed that *R. philippinarum* is expected to have moderate growth in length or individual body mass (ultimate length and body weight would be larger than current values by 2–3%) when taking into account only the effect of temperature increase. However, acidification is likely to have a deleterious effect on growth, with a decrease of 2–5% length or body weight under the pH value of 7.7 forecasted for the end of the 21st c, or 10–15% under a more extreme scenario (pH = 7.3). However, the aggregated reproductive potential, integrated along a lifetime of 10 years, is likely to increase by 30% with temperature increase. Decreasing pH would impact negatively on reproductive potential, but in all simulations under warmer conditions, reproductive potential values were higher than current, suggesting that temperature increase would compensate losses due to acidification. The results are discussed in relation to their possible impact on aquaculture and fisheries of this important commercial bivalve.

1. Introduction

Among the environmental changes forecasted for the world's climate over the current century, further increases in water temperature and acidification are expected in the ocean, as a result of land-based human activities (IPCC, 2019). In marine ectotherms, synergies between temperature increase and acidification have been documented (Pörtner and Farrell, 2008; Lannig et al., 2010), compounding the problem of exposure to global change. Marine bivalves are particularly vulnerable to these physical stressors because, being benthic ectotherms, their biology and physiology will most likely be affected by seawater temperature increase and acidification, which may impair physiological processes related to shell calcification among others (Matozzo and Marin, 2011; Gosling, 2015; Munari et al., 2018). Several effects on the well-being of bivalves subjected to increased temperature and lowered pH have been reported in the literature including modifications in growth rates, increased immunological response and pathologies, and altered metabolic rates related to energy production, oxidative stress and detoxification processes (Range et al., 2014; Matozzo and Marin, 2011; Nardi et al., 2018; Liao et al., 2018; Magalhaes et al., 2018; Costa et al., 2020). For example, it has been shown that filtration, ingestion and oxygen-consumption rates of the clam *Ruditapes philippinarum* (Adams and Reeve, 1850) increased with temperature (Kyung et al., 2008), while acidification resulted in reduced metabolic rates in the clam *Ruditapes decussatus* (Range et al., 2014). These metabolic modulations may, in turn, alter the relationship between clams and their environment, i.e., increasing or diminishing their filtration and thus the rate of phytoplankton grazing. Moreover, the effects that high temperature and low pH are capable of inducing may be enhanced in combination with other stressing factors, such as contaminants. For instance, Munari et al. (2018) tested the effect of exposure to increasing concentrations of the drug diclofenac under reduced pH conditions and found that the damage endured by *Mytilus galloprovincialis* and *R. philippinarum* from acidification was compounded in some cases, by the exposure to the pharmaceutical drug.

Marine bivalves are ecologically important components of coastal

☆ Temperature increase and acidification in *Ruditapes philippinarum*.

* Corresponding author.
E-mail addresses: maynouf@icm.csic.es (F. Maynou), galimany@icm.csic.es (E. Galimany), mramon@icm.csic.es (M. Ramón), msole@icm.csic.es (M. Solé).

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ecosystems because of their provision of services, such as nutrient bioremediation in eutrophic waters and habitat-forming capacity, which favors biodiversity (Smaal et al., 2019). They are also important to coastal economies that commercially exploit bivalve beds, either as small-scale fisheries or aquaculture operations (Gosling, 2015). Therefore, seawater temperature increase and acidification will likely hamper the provision of goods and services mediated by bivalves, especially in areas more vulnerable to climate change such as the Mediterranean Sea. Climate model projections predict that temperature and acidification in the Mediterranean may be more drastic than forecasted global averages. In fact, the Mediterranean Sea is considered a 'hotspot' for climatic change (Diffenbaugh and Giorgi, 2012). Moreover, future environmental conditions are likely to reinforce the trends already detected for the past 2-3 decades, including sea surface temperature increase by ca. 0.5 °C in the period 1980–2000 and pH decrease by 0.1 units reported in recent decades (Calvo et al., 2011; Durrieu de Madron et al., 2011). Model projections show increases in sea surface temperature of up to 2.5 °C by the end of the century under the future warming scenario named RCP8.5 ("business as usual") and a further decrease by 0.4 pH units is expected at the end of the century (Hartin et al., 2016). Understanding the responses of bivalves to future environmental conditions is essential to strategically preserve and manage bivalve beds (Smaal et al., 2019). Dynamic Energy Budget (DEB, Kooijman, 2010) theory captures the metabolic processes of an organism through its entire life cycle as a function of temperature and food availability. The DEB model is a tool that can be used to model lifetime feeding, growth, reproduction, and their responses to changes in biotic and abiotic conditions. Modelling the quantification of energetic demands of organisms under variable levels of stress (Flye-Sainte-Marie et al., 2009; Anacleto et al., 2018; Mónaco and McQuaid, 2018) is a valuable tool to help understand the effects of environmental conditions on the metabolism of organisms. In particular, several studies applying DEB models have proved to be a useful tool to assess the effects of climate stressors on growth and reproduction of different species of bivalves (Mónaco and McQuaid, 2018, 2019; Chowdhury et al., 2018; Steeves et al., 2018).

Ruditapes philippinarum is a non-native clam species well established in the Mediterranean Sea and in the Atlantic waters south of the English Channel (Range et al., 2014; Flye-Sainte-Marie et al., 2007, 2009). It was introduced in Europe from the western Pacific Ocean in the early 1970s for aquaculture purposes because of its tolerance to a wider range of temperatures and higher growth rates than the native clam *R. decussatus* (Flye-Sainte-Marie et al., 2009). Currently, its abundance in the coasts of the Iberian Peninsula may be comparable to, and locally be even larger than, that of *R. decussatus* (Juanes et al., 2012; Velez et al., 2015). Increasing sea water temperature may favor the expansion of non-native species such as *R. philippinarum*, given its faster growth, higher fecundity and longer spawning period than native clams (Moura et al., 2018). However, some studies with other bivalve species suggest that future climate change conditions may disfavor non-native species. For example, DEB model studies on two intertidal mussel species inhabiting South Africa shores, the native *Perna perna* and the invasive *Mytilus galloprovincialis*, concluded that the reproductive potential of both species is likely to increase under future temperature forecasted scenarios (Mónaco and McQuaid, 2018, 2019). But the effect of elevated temperature on reproduction was less significant for *M. galloprovincialis* than for *P. perna*, suggesting that under future environmental conditions the expansion potential of the invasive bivalve species may be compromised. These results highlight the interspecific variability of bivalves and the need to study the species independently to better predict changes in abundance and the consequences on ecosystem services, fisheries, and aquaculture.

The objective of the present study is to assess the effect on key energy demanding processes such as growth and reproductive potential of enhanced temperature and acidification estimated to occur under a climate change scenario on the commercial clam, *R. philippinarum*, and evaluate this energetic cost by using the DEB model.

2. Material and methods

2.1. Experimental animals

Seed-size clams *Ruditapes philippinarum* were purchased from Satmar, the Marennes nurseries, France. Animals were acclimated to laboratory conditions at room temperature and natural filtered sea water for one week before the start of each of the four seasonal experiments. Laboratory experiments evaluating the effects on growth and clearance rates of the juvenile clams under variations in temperature and pH were carried out, both independently (analyzing temperature and pH separately) and in combination (analyzing both factors together).

2.2. Experimental set up

The laboratory experiment was set in the Zone of Aquaria and Experimental Chambers (ZAE) at the Institute of Marine Sciences (ICM-CSIC). Briefly, 35 clams were placed in 20 L glass aquaria (all treatments were run in triplicate) with a flow through system with filtered (porosity: 50, 10 μm and 1 μm) sea water (Fig. S1 of Supplementary Material provides a schematic of the experimental setup). Clams (approx. 9–15 mm shell length, ShL) were continuously fed a 1:1 mixture of two phytoplankton species, *Isochrysis galbana* and *Tetraselmis* sp. at a density of 4.0×10^6 cells L^{-1} using a peristaltic pump. The clams were acclimated to the experimental conditions lowering the pH by 0.1 units daily until de target pH was reached. Aquarium thermostat heaters were used to increase the temperature by 1 °C every 2 days until final experimental conditions. Then, clams were exposed for 4 weeks to seasonal experimental temperatures and pH. The experiments were done in flow-through systems to avoid accumulation of waste products that might interfere with the pH treatments.

Four seasonal experiments were conducted, mimicking natural conditions in the Ebro Delta bays, an important bivalve aquaculture area in the Western Mediterranean (Ramón et al., 2009). The experiments were carried out in May 2018 (spring), July 2018 (summer), October 2018 (autumn) and January 2019 (winter). Two temperature x 3 pH scenarios were tested. The current (early 21st c.) average ambient temperatures in the Ebro delta bays ranges from a min. of 6 °C in January to a max. of 30 °C in August, with climatological values of 10–28 °C (Ramón et al., 2007; Llebot et al., 2011). For the experiments the ambient temperatures were: spring: 15 °C, summer: 23 °C, autumn: 18 °C, winter: 14 °C, taken as representative of average surface water temperature in the Ebro Delta bays in each season (Fig. 1). The warmer water treatment consisted in increasing the ambient water temperature

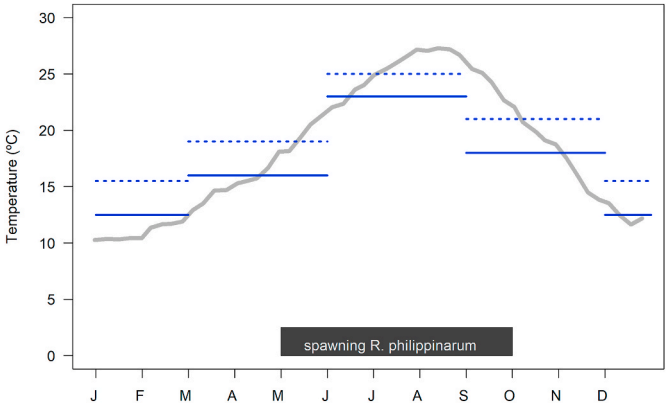

Fig. 1. Simulation conditions along one year with observed climatological temperature in the Ebre delta bays (Llebot et al., 2011). Grey continuous line: average weekly temperature observed in the study area (1993–2004). Continuous blue line: experimental ambient temperature; dotted blue line: experimental warmer temperature regime (+2/3 °C). The black box indicates the spawning period of *Ruditapes philippinarum* (May to September).

by 3 °C in spring, autumn and winter and 2 °C in summer, using a water bath with heaters (see schematic in Fig. S1). The heaters were programed and controlled by a sensor that turned them on immediately after the temperature from the water bath decreased. Technical limitations precluded increasing the summer temperature by 3 °C, as in the other seasons. The three pH levels were 8.1 (neutral), 7.7 and 7.3, which were adjusted by bubbling CO_2 into the experimental water. pH measurements were controlled with glass electrodes calibrated on a daily basis with a TRIS buffer, following standard procedures (SOP6a of Dickson et al., 2007). The rationale for these values is the projected temperature increase at the end of the century under RCP8.5 (IPCC, 2019) and the projected pH decrease of 0.4 units (7.7) for the world ocean (Hartin et al., 2016), plus a more extreme acidification case (7.3). In the study area pH averages 8.1, as in the adjacent Mediterranean Sea, but varies from 7.9 to 8.3 along the seasonal cycle (Prado et al., 2016).

2.2.1. Growth

Growth rates were calculated as changes in shell length and total weight of the juvenile clams, which were measured at the beginning and end of the experiment. Shell length was measured (maximum anteroposterior distance) to the nearest 0.01 mm with a Vernier caliper, and total weight was obtained with an electronic scale (±0.001 g). Clam sizes were equally distributed among the several aquaria conditions and replicates. A summary of our experimental results on growth used to help parameterize the DEB model is given in Supplemental Table S1 (ambient temperatures) and S2 (predicted increased temperatures). Statistical differences in growth among seasons and experimental conditions (3-way ANOVA: season x temperature x pH) are reported in Table S3.

2.2.2. Clearance rates

Clearance rates, i.e. the volume of water cleared by clams per unit of time (L h^{-1}), were estimated by quantifying the decrease in algal cell density (1:1 *Tetraselmis* sp.:*Isochrysis galbana* at a concentration of 4 × 10^3 cells mL^{-1}) from the experimental water. Five groups of 3 clams per treatment were used for each measure, i.e. fifteen clams for each combination of temperature and pH were analyzed. Each of the three clams per group were collected from a different replicate tank (Fig. S1) and was placed at the bottom of 50 mL test-tubes previously cut with several openings all around the tube allowing water to flow through. The clams in the tube were immediately placed in a 250 mL glass bottle with 200 mL of experimental water from the aquaria. The tubes with clams were maintained elevated and stable in the glass bottle using tin wire to allow the placement of a small magnet underneath. The bottles were then placed on a multi-position magnetic stirring plate to ensure resuspension of particles. The reduction in the number of particles as a function of time was monitored by taking 10 water samples during 1 h. Each collected water sample was analyzed for particle concentration analysis using a BD Biosciences Flow Cytometer (San Jose, California). Then, clearances rates were determined using the formula (Riisgard, 2001):

$$CR \cdot = \cdot \left(V/n_t\right) \cdot \ln \cdot \left(C_0/C_t\right)$$

where C_0 and C_t are algal concentration at time 0 and time t, V is the volume of water, and n_t is the number of individual clams. All CR values were standardized to 1 g of dried clam to allow comparisons (Supplemental Tables S1 and S2).

2.3. DEB model

A simulation model based on Dynamic Energy Budget (DEB, Kooijman, 2010) was built to assess the growth and reproductive potential of an ideal *R. philippinarum* individual along its lifetime. We choose to use the DEB modelling framework owing to its ability to model underlying physiological processes (growth, reproduction, and maintenance) based on first principles that are applicable across different taxa and ontogenetic stages and the high quality of existing parameter sets in the literature (Sousa et al., 2010; Monaco et al., 2014). We examined the performance of *R. philippinarum* under 2 temperature and 3 pH scenarios. Following other applications of the DEB theory to bivalves (e.g. Flye-Sainte-Marie et al., 2009; Mónaco and McQuaid, 2019), we adopted the *abj* DEB model (i.e. assuming the equations of a standard animal with acceleration growth between birth and metamorphosis, Kooijman, 2010). In this extension of the standard model, the life cycle of an animal is divided into four main stages: embryo (non-feeding stage), larva (feeding, non-reproductive stage), juvenile (feeding, non-reproductive stage), and adult (feeding, reproductive stage). The stages are separated by the birth, metamorphosis and puberty transformations. In the *abj* model then, the juvenile stage is divided in two phases: larva (pre-metamorphosis) and juvenile *stricto sensu* (post-metamorphosis) to account for differences in growth along this stage.

At each stage, the energy (or mass) of an individual can be apportioned in three compartments: structure (somatic tissue, *V*), reserves (*E*) and maturity (*H*) (Kooijman et al., 2008). Energy ingested from food is first allocated to the reserve compartment and the flux of energy from reserves is transferred to somatic maintenance + growth and to maturity + reproduction buffer, according to a constant ratio κ ("kappa-rule"). Reproduction buffer is a parameter based on the accumulation of invested energy for reproduction, which empties after each spawning. DEB models give priority to maintenance, drawing energy from the reproduction buffer or structure if maintenance costs cannot be met with ingested food. The Electronic Supplementary Material provides details on the DEB model.

The energy budget model for *Ruditapes philippinarum* was parameterized with values obtained from add_my_pet database for this species (https://www.bio.vu.nl/thb/deb/deblab/add_my_pet; Flye-Sainte-Marie et al., 2009) as starting values. The actual parameter values (Table 1) used for the DEB simulation model were a combination of literature parameters (chiefly Flye-Sainte-Marie et al., 2009) and parameter estimates from growth and feeding rates of the individuals in our experiments obtained using the covariation method (Lika et al., 2011) available in the MATLAB routines of the DEB model tool (DEBtool_M, http://www.bio.vu.nl/thb/deb/deblab). For each species in the add_my_pet collection, the DEB model tool is tailored with observed data (from other authors' experiments) and parameters, which were be used as initial parameter estimates in our application. In effect, although physiological parameters will vary among individuals of the same species and populations, it can be assumed that the variation of parameter values within taxa is lower than the variation across taxa (Kooijman et al., 2008). Hence, the parameter estimates for the DEB model were initialized with the parameter values from the add_my_pet database. The MATLAB routines were run for 1000 times under each pair of experimental conditions to compute the mean and standard deviation when possible. Only for the fluxes $\{\dot{p}_{Xm}\}$ and $[\dot{p}_M]$ was it possible to estimate variable values under different experimental conditions.

Simulations were carried out with an adaptation of the R code made available by Monaco and McQuaid (2019) at https://github.com/cristianmonaco/DEB-model-application-Mussel-zonation-and-reproductive-potential. Our four simulation scenarios were: 1) current temperature and pH regime (seasonal averages for the second decade of the 21st c.), 2) temperature increase, as in the experimental regime (3 °C spring, autumn and winter; 2 °C summer) with current pH, 3–4) current and increase temperature, respectively with pH = 7.7, and 5–6) current and increase temperature, respectively with pH = 7.3. Fig. 1 summarizes the simulation conditions along one year.

The simulations were carried out for 10 years at daily scale (3650 time steps, *t*), with 1000 iterations based on the mean and standard deviations of the variable fluxes $\{\dot{p}_{Xm}\}$ and $[\dot{p}_M]$. At each time step the value of the fluxes and state variables in Table S4 were computed. Considering that *Ruditapes philippinarum* spawns between May and September in the Mediterranean Sea (Range et al., 2014) and also in the

Table 1
Parameters of the DEB model for *Ruditapes philippinarum* based on values from add_my_pet, Flye-Sainte-Marie et al., (2009) and values estimated here with the MATLAB DEBtool_M routines. For $\{\dot{p}_{Xm}\}$ and $[\dot{p}_M]$the average ± SD are shown. Refer to Supplemental Table S4 for the symbols. All rates given for a reference temperature T_{ref} of 20 °C (293.15 K).

	pH 8.1	pH 7.7	pH 7.3
$\{\dot{p}_{Xm}\}$ (J d^{-1} cm^{-2}) spring	3.772 ± 0.163	3.779 ± 0.130	3.769 ± 01.51
summer	3.740 ± 0.156	3.770 ± 0.157	3.724 ± 0.158
autumn	4.273 ± 0.132	3.759 ± 0.156	3.774 ± 0.166
winter	3.752 ± 0.142	3.774 ± 0.135	3.770 ± 0.161
$[\dot{p}_M]$ (J d^{-1} cm^{-3}) spring	9.728 ± 0.159	11.106 ± 0.169	9.699 (0.178)
summer	9.708 ± 0.167	9.719 ± 0.146	9.736 ± 0.191
autumn	11.118 ± 0.156	9.756 ± 0.199	9.703 ± 0.181
winter	11.099 ± 0.170	11.114 ± 0.173	9.744 ± 0.155
d_V (g cm^{-3})	0.100		
δ_M	0.427		
y_{VE} (J J^{-1})	0.976		
$\dot{\nu}$ (cm d^{-1})	5.10×10^{-3}		
$\dot{k}_J$ (d^{-1})	1.80×10^{-3}E-03		
κ	0.992		
κ_R	0.950		
g	4.0314		
$[E_G]$ (J cm^{-3})	2357.884		
ρE (g J^{-1})	1.82×10^{-6}		
L_b (cm)	0.003		
L_j (cm)	0.019		
L_p (cm)	0.715		
L_i (cm)	2.046		
a_b (d)	2.409		
a_j (d)	18.436		
a_p (d)	399.984		
a_m (d)	2059.800		
E_0 (J)	6.71×10^{-5}		
E_H^b (J)	4.48×10^{-7}		
E_H^j (J)	$1.34\ 10^{-4}$		
E_H^p (J)	8.885		
T_A (° K)	6071		
T_L (° K)	275		
T_H (° K)	300		
T_{AL} (° K)	30424		
T_{AH} (° K)	299859		

Sea of Japan (Ngo et al., 2018), although the spawning period can extend to November–December in Portugal (Moura et al., 2018), we initiated the simulations at t = 1 corresponding to 1st October under "autumn regime". Individuals in the model were allowed to growth when $\kappa \cdot \dot{p}_C > \dot{p}_M$, i.e. the fraction of mobilized reserve was larger than the maintenance costs. Investment in reproduction occurred when (1-κ) $\cdot \dot{p}_C > \dot{p}_J$, i.e. the fraction mobilized for reproduction or maturity was larger than the costs of maturity maintenance. Spawning (emptying the reproduction buffer in the model) occurred from days 240–365 of each year (i.e. 1st May to 30 September).

3. Results

Growth varied significantly in the four seasonal experiments, depending on temperature and acidification conditions (Tables S1, S2, S3 of the Supplementary Material). In brief, significant differences in growth were detected by season, temperature and pH, as well as their pairwise interactions. Growth was relatively high in winter and spring (average 0.0256 mm/d). Growth in summer and autumn was very low (average 6×10^{-5} mm/d) under all experimental conditions. Furthermore, in spring and autumn warmer temperatures generally resulted in higher growth (post-hoc Student-Newman-Keuls test, Table S3). More acidic conditions resulted in lower growth, especially in summer and autumn, but note that the highest growth rates were achieved in winter at both reduced pH levels (Tables S2 and S3). The interaction between temperature and pH showed that ambient temperature and control pH tended to yield the lowest growth, across seasons, while warm conditions and control or moderately reduced pH estimulated higher growth (Table S3).

The DEB model parameters estimated for *Ruditapes philippinarum,* under our experimental conditions, are shown in Table 1. The ingestion rate $\{\dot{p}_{Xm}\}$ varied between 3.74 and 4.27 J d^{-1} cm^{-2} depending on the physical parameters of the rearing conditions, while the somatic maintenance rate $[\dot{p}_M]$ ranged from 9.70 to 11.12 J d^{-1} cm^{-3}. For all other parameters no meaningful variability could be estimated. The values of κ (0.992) and g (4.031) obtained here were larger than the corresponding values in the same parameter collection reported by Flye-Sainte-Marie et al. (2009) (0.890 and 1.384, respectively). The remaining DEB model parameters were not changed with respect to their starting value during the parameter estimation process.

Fig. 2a shows that growth (in physical length) was faster under warmer than under ambient conditions, particularly for the first four years of the simulation (until day 1400 approximately). The largest ultimate lengths (i.e. the ceasing of growth) for scenarios under pH 8.1 were predicted at 4.59 cm ShL under ambient temperature and 4.63 cm ShL in the warmer conditions (Table 2). By contrast, the effect of pH revealed slower growth rates with decreasing pH for both ambient and warm scenarios with largest ultimate lengths predicted for pH 7.3 at 4.37 cm and 4.43 cm under ambient and warm conditions respectively (Table 2). Likewise, ultimate dry body mass was systematically larger under warmer conditions (Fig. 2b), but lower pH had a detrimental effect on growth and ultimate dry body mass (Table 2). Similarly, to shell length results, the largest ultimate dry body masses were accomplished under pH 8.1, i.e. 0.76 g for ambient temperature and 0.78 g under the warming scenario. However, at pH 7.3, the largest ultimate dry body masses lowered to 0.66 g and 0.69 g under ambient and warm temperatures respectively.

The reproductive buffer, energy invested for reproduction, was also larger under warmer conditions (Fig. 2c) and decreased with more acidic conditions (Table 2); that is, the highest value was found at warm water and pH 8.1 (137.09 J $clam^{-1}$) and the lowest at ambient temperature and pH 7.3 (97.58 J $clam^{-1}$). Note, however, that the cumulative reproductive output under warmer conditions was always larger than under ambient conditions, regardless of pH (Fig. 2d). That is, under warmer conditions, even the lowest pH resulted in higher reproductive output. Cumulative individual reproductive output was greater than 125 kJ $clam^{-1}$ at warm conditions, while it was below 110 kJ $clam^{-1}$ for all ambient situations. In terms of number of eggs, the total production was equivalent to 3.15×10^6 eggs $clam^{-1}$ under current conditions (ambient temperature, pH 8.1) and 3.82×10^6 eggs $clam^{-1}$ under forecast parameters (warmer temperature, pH 7.7).

4. Discussion

The results of the experiments at ambient temperatures and control pH 8.1 showed seasonal variations in the growth of *Ruditapes philippinarum*, with higher growth rates in spring and winter than in summer or autumn. Moreover, the experimental growth rates under warmer conditions, at control pH, showed significantly higher growth rates (cf Tables S1 and S2), which can be expected given the original subtropical habitat of this non-indigenous species in European waters. The experimental effects of pH under ambient temperatures were contrasted, with reduced growth rates at increasingly reduced pH in spring, but increasing in winter (Table S1). A similar pattern was apparent under the warm experimental regime (Table S2). Note that in summer and autumn the growth rates were so low that the differences among experimental conditions, even if statistically significant, would have practically no consequences on individual growth. Translating these experimental results into metabolic parameters and building a Dynamic Energy Budget (DEB) model helped understand the impact of varying growth and feeding rates due to increase temperature and decreased pH at population level.

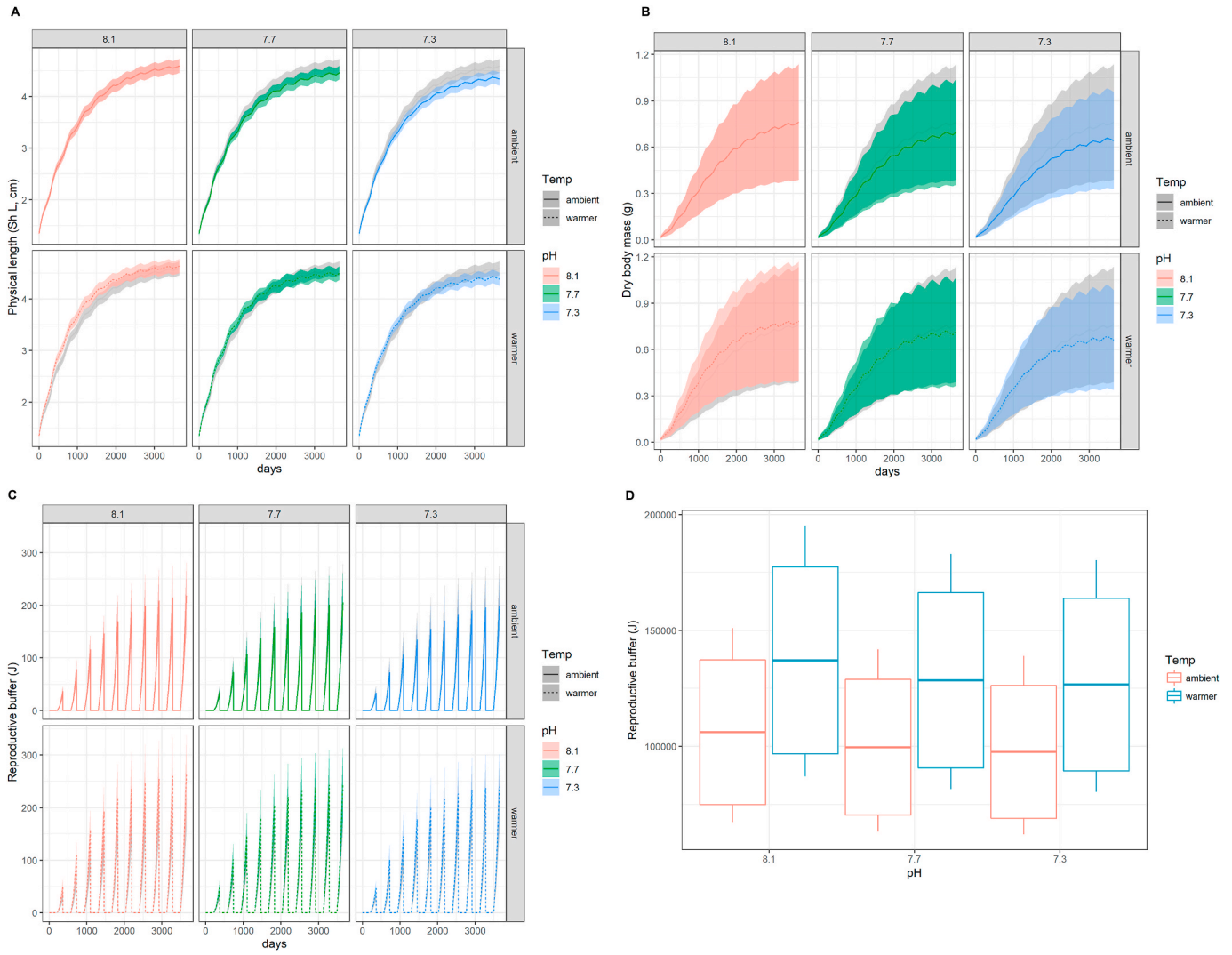

Fig. 2. Results projected of the application of the DEB model to *Ruditapes philippinarum* along a 10 year simulation period. The panels show at top left the current, or control, conditions (ambient temperature, pH = 8.1) and the remaining 5 panels the different environmental conditions for projection. Note that the control conditions are repeated as grey background for each panel.

Table 2
Summary results of predicted shell length, body mass, and aggregated reproductive potential within 10 years. The current scenario (beginning of 21st c.) is represented by the ambient temperature and pH 8.1, while the conditions at the end of the 21st c. are projected to be warmer by 2–3 °C and pH 7.7

pH	Temp	ultimate shell length (cm)	ultimate dry body mass (g)	aggregated reproductive potential along 10 years (J clam^{-1})
8.1	ambient	4.591	0.762	106,072
7.7	ambient	4.461	0.699	99,615
7.3	ambient	4.373	0.658	97,580
8.1	warmer	4.633	0.784	137,089
7.7	warmer	4.509	0.722	128,478
7.3	warmer	4.433	0.686	126,649

Following the seasonal sea water cycle from an aquaculture region in the NW Mediterranean Sea, the clam *Ruditapes philippinarum* can be predicted to grow moderately larger under a climate warming scenario (of 2–3 °C increase) expected by the end of the 21st c. Several studies have shown that marine invertebrates, including bivalves, respond positively to increased temperature, up to the limit when increased metabolic costs cause thermal stress and may hamper growth (Galimany et al., 2011; Steeves et al., 2018). However, the effect of acidification, as seen in our model results, is likely to be consistently detrimental to growth, with progressively smaller ultimate length or body mass with decreasing pH. Under the conditions forecasted for the end of the 21st c. in the Mediterranean Sea, that is 2-3 °C warmer temperature and pH = 7.7, our results predict that ultimate shell length will decrease by 2% and the decrease in body mass is projected to be even larger by 5.5%. Fernández-Reiriz et al. (2011), testing the same values of pH than in our study in the native Mediterranean clam, *R. decussatus*, also reported growth impairment in juveniles, seen as decreased metabolic and increased excretion rates. The deleterious effect of reduced pH on the growth and survival of larval and juvenile stages of other bivalves, including mussels and oysters, has been documented with laboratory experiments, validating the results of the model (Range et al., 2011; Kong et al., 2019; and references within).

Projecting bivalve growth and biological performance is relevant to predict shellfish ecosystem services and biomass extraction in future aquaculture practices and fisheries. Bivalves play a very important role in the ecosystems maintaining water quality when controlling phytoplankton biomass by grazing. For example, in South San Francisco Bay, bivalves could filter a volume equivalent to the volume of the bay at least once daily (Cloern, 1982). However, changes in bivalve populations, i.e. growth, filtration capacity or abundance, may compromise

the ecosystem service of water quality enhancement. For example, after a massive recruitment of the clam *Mya arenaria* in Ringkøbing Fjord, Denmark, the excess of benthic grazing became the key modulator of the biological structure, causing a sudden drop in chlorophyll *a* concentration from 64.6 μg L^{-1} in 1995 to 21.0 μg L^{-1} and 7.6 μg L^{-1}, in 1996 and 1997 respectively (Petersen et al., 2008). On the contrary, when bivalve populations decrease, the reduction of grazing pressure may enhance harmful algal blooms equally disrupting functional ecosystems (Buskey et al., 1997). The filtration capacity of bivalves not only has an uttermost importance on phytoplankton composition and abundance but it also offers other ecosystem services by controlling the availability of nutrients in the water column and benthic organisms. As an example, studies of *R. philippinarum* in Sacca di Goro Lagoon (Italy) revealed that the clams contributed with a 64–133% of the total rates of sediment oxygen uptake, nitrogen regeneration, nitrification, and denitrification indicating that clam biomass plays a key role in nitrogen cycling in bivalve farming areas (Welsh et al., 2015). Therefore, the reported lower growth due to acidification in the projections of this study implies a decrease of filtration capacity, as gill size is proportional to squared length in bivalves, thus a loss of water quality maintenance and nutrient transfer.

Aside from the ecosystem services that bivalves may provide, *R. philippinarum* is a valuable commercial seafood that has a global aquaculture production of over 4.2 Mt a year (FAO FishStat). In the European Mediterranean and the Black Sea, the production in 2017 was about 37.45 t with a value of 226 M€ (FAO Stats https://ec.europa.eu/eurostat/data/database). Therefore, any relevant biological parameters, including the reproductive potential, which is the relative capacity of a species to reproduce under optimum conditions, will be targeted to estimate maximization of clam production. In our study, the reproductive potential is forecasted to be enhanced under warmer conditions, as it could be anticipated, given the positive relationship between reproductive output and temperature in bivalves and other marine invertebrates (Gosling, 2015), although up to a certain limit. In our study, the cumulative reproductive potential of *R. philippinarum* is expected to increase by 21% between the current conditions (ambient temperature and pH 8.1) and the likely environmental conditions at the end of the 21st c (warm temperature, pH 7.7). Range et al. (2014) experimentally showed that long-term exposure for several months to acidic conditions severely damaged the shells of some bivalve species (*Mytilus galloprovincialis* and *Chamelea gallina*) and decreased the metabolism in *R. decussatus*, but specific studies on the effects of pH on reproduction are scarce. Range et al. (2011) showed that under acidic conditions (pH 7.7) spawning was delayed in *R. decussatus* as a trade-off to survival, while Xu et al. (2016) concluded that spawning capacity may be impaired under acidification in *R. philippinarum*. Other authors have experimentally revealed that larval survival and growth were impaired in *Donax trunculus* at lower pH (Pereira et al., 2016). Conversely, a study by Fernández-Reiriz et al. (2012) found that the growth of individuals of a population of *M. galloprovincialis* was actually favored in a more acidic media and concluded that the existence of ecotypes resistant to pH decreases may aid this mussel to cope with the forecasted conditions for oceans at the species level. The possible intergenerational adaptation to increased acidity should also be taken into account when applying DEB models at a population level. This was highlighted at least for the highly invasive mussel *Musculista senhousia*, when exposure to low pH levels for several generations led to rapid acclimation to changing conditions, even resulting in increases in reproductive fitness (Zhao et al., 2019). However, Kong et al. (2019) could not demonstrate intergenerational adaptation to reduced pH in the edible mussel *Mytilus edulis*. The present DEB modelling approach cannot, by its very nature, tackle the problem of high mortality of bivalves due to episodic events of extremely high temperatures during the summer period (Ramón et al., 2007), which are likely to increase its frequency along the 21st c. with climatic change (Darmaraki et al., 2019) or other exogenous factors that may limit aquaculture productivity.

Our study confirms that controlled experiments considering future environmental conditions can help parameterize a complex DEB model aimed to forecast the effect on growth and reproductive output of marine species. Nonetheless, the present application has some limitations as we evaluated the response to temperature and pH stressors on juveniles (0.9–1.5 cm ShL range approximately) and other important parameters implied in reproductive performance, such as quality of spat or quality of shell formation, could not be assessed. Future studies need to address these and other modulators of biological performance, such as the metabolic cost of parasite loads or exposure to pollutants, that can synergistically affect natural populations under climate change scenarios (Flye-Sainte-Marie et al., 2009; Rowley et al., 2014; Prado et al., 2016; Munari et al., 2018; Anacleto et al., 2018).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2020.107099.

A: Shell Length (cm); B: Dry body mass (g); C: Reproductive potential (J); D:. Cumulative reproductive potential along a 10-year simulation period (J/clam).

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